targets with CAR-MUC1 T cells, in the presence of IL2 for 72h, and found that >60% of CAPAN1 and >40% of DU145 were killed after an initial treatment but were resistant to subsequent treatment. Importantly IHC analysis of these resistant tumor cells demonstrated lack of expression of the targeted antigen, MUC1. This observation was confirmed in an artificial model using 293T cells engineered to express either MUC1 (mOrange+) or PSCA (GFP+), which illustrated an exquisite specificity of T cells but at the same time a weakness when targeting a heterogeneous tumor cell population. Therefore we generated a second CAR targeting the TAA PSCA, and demonstrated stable expression on primary T cells (89±2%), which were able to kill the PSCA+ cell lines, CAPAN1 and DU145, with no effect on control PSCA- 293T, (48±6%, 41±46% and 4±2% specific lysis, respectively, 10:1 E:T). Finally, we assessed the anti-tumor effects when both CAR-modified products were combined. When tumor cells expressing both TAAs were treated with CAR-MUC1 + CAR-PSCA T cells we saw additive anti-tumor effects with 76±10% killing of CAPAN1 compared with only 35±6% and 48±6%, respectively, using CAR-MUC1 and CAR-PSCA T cells individually. We saw similar results using our engineered 293T tumor model. Hence the combination of CARs that target two distinct TAA expressed on cancer cells (PSCA and MUC1) may prevent tumor immune escape and enhance the potency of adoptive T cell transfer.

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### Peri-Transplant Toxicity for Pediatric Patients with High-Risk Medulloblastoma Undergoing Tandem Autologous Hematopoietic Cell Transplantation

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**Background:** Medulloblastoma is the most common brain tumor in children and its prognosis is improved by the combination of surgical resection, irradiation, and/or chemotherapy. Chemotherapy followed by autologous peripheral blood stem cell (auto-PBSC) rescue has been used for the treatment of high-risk medulloblastoma.
**Patients & Methods:** Between July 2010 and July 2012, 8 patients with high-risk medulloblastoma underwent tandem chemotherapy with stem cell rescue procedures implementing one of two protocols. Four children aged less than 36 months old [median age 30 month old (range 24-33 months old)] (group-A) received the Children's Oncology Group – ACNS0334 protocol. The other 4 patients aged more than 36 months old [median age 12 year old (range 9-15 years old)] (group-B) were treated according to the St. Jude - SJMB03 protocol.
**Results:** All patients engrafted well and are alive with no evidence of disease. Median follow-up from discharge for last transplant is 6.5 months (range 3-24 months). Neither cardio nor pulmonary toxicities were documented. Gastrointestinal tract: No patient had veno-occlusive disease of the liver. All patients developed mild to moderate mucositis, thus received total parentral nutrition for a median period of 7 days (range 5-12 days). Infections: group-A: all patients experienced at least 1 transplant period without any fever. Bacteremia included staphylococcus Coa. (-), proteus sp. and pseudomonas sp. Neither viral nor fungal infections were documented. Group-B: 2 patients experienced at least 1 transplant without any fever. Bacteremia included staphylococcus Coa. (-), staphylococcus aureus and pseudomonas sp. Metabolically: 2 group-B patients developed inappropriate anti-diuretic-hormone secretion. All patients developed mild hypomagnesaemia. Neurology: 1 group-B patient developed generalized motor weakness attributed to MESNA he received for the cytoxan. From then on, cytoxan was given without MESNA but with urinary catheterization and hyperhydration. He also developed mastication movements attributed to the resperidal he got, which was stopped with improvement. No patient developed major bleeding. Renal: 1 group-A patient received VP-16 instead of carboplatine on the first transplant due to basic renal impairment. 1 group-B patient suffered from renal impairment with GFR reduction between 25%-50% of baseline following his first transplant. He also demonstrated grade-3 ototoxicity and thus received only 50% cisplatin dose from the third transplant. Another 2 group-B patients suffered from grade 4 ototoxicity before admission and therefore did not get cisplatin at all.
**Conclusions:** Tandem auto-PBSC transplants are feasible for high-risk medulloblastoma pediatric patients, with excellent engraftment and survival results and acceptable toxicities, applying the ACNS0334 and SJMB03 protocols.

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### Anti-Tumor Effects of Chimeric Receptor Engineered Human T Cells Directed to Tumor Stroma

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Adoptive T-cell immunotherapy for solid tumors though promising has shown limited efficacy in clinical studies. The hindrance to achieving an objective and durable therapeutic response is, in part, mediated by the dynamic nature of the tumor and its complex microenvironment. Tumor-directed therapies fail to eliminate components of the microenvironment, which can reinstate a tumorigenic milieu and contribute to recurrence. Cancer associated fibroblasts (CAFs), the most preponderant cell type in the microenvironment, contribute to desmoplasia, tumor growth and therapeutic resistance. CAFs express fibroblast activation protein alpha (FAP), a membrane bound serine protease, in a number of a solid tumors making it an attractive immunotherapeutic target. We hypothesized that targeting CAFs with FAP-specific T cells will destroy the 'tumor promoting haven', resulting in significant anti-tumor effects.
To test this hypothesis, we successfully generated FAP-specific T cells using a second-generation chimeric antigen receptor (CAR) specific for FAP. A prototypical CAR combines the antigen specificity of an antibody with the signaling function of a T-cell. The resulting genetically engineered FAP-specific T cells recognized and killed human as well as murine FAP-positive target cells ex vivo. FAP-specific T cells also led to a significant decrease of FAP-positive murine lung stromal cells with a concomitant reduction in A549 lung tumor growth and improved survial when administered systemically into SCID mice. Targeting FAP-positive CAFs alone, therefore induces a significant anti-tumor response indicative of its important role in tumor progression.
Finally, given the reciprocal relationship between tumor cells and CAFs, we hypothesized that co-targeting these two compartments would result in enhanced anti-tumor response

than targeting either alone. Erythropoietin-producing hepatocellular carcinoma-A2 (EphA2)-specific CAR T cells were used to target the A549 tumor cells. EphA2-specific T cells when administered together with FAP-specific T cells, resulted in a significant decrease in tumor growth and increased survival compared to mice that received either EphA2- or FAP-specific T cells alone. Our study underscores the value of co-targeting both CAFs and cancer cells to increase the benefits of T-cell immunotherapy for solid tumors.

**POSTER SESSION 2: STEM CELL BIOLOGY**

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### Fully Automated Clinical-Scale Separation of CD133$^+$ Cells From Bone Marrow Aspirate

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There is a growing interest in CD133 antigen expressing stem cell in the field of regenerative medicine such as cardiovascular, peripheral artery, and liver disease. Investigators in particular concentrate on bone marrow-derived stem cells for these applications. Today the clinical scale enrichment of CD133$^+$ cells has to be performed as a complex procedure involving numerous manual handling steps.

We have developed a fully automated clinical scale process within a closed sterile system to purify CD133$^+$ cells from human bone marrow aspirates. In this context, erythrocyte reduction, generation of autologous plasma, labeling time and the conditions for immunomagnetic separation were optimized.

To determine the process performance, CD133$^+$ cells were separated from human bone marrow aspirates with an initial volume of about 60 mL (n=10). We performed colony-forming unit (CFU) assays, which allowed us to evaluate the differentiation potential of the enriched cells.

The total processing time was reduced from about 4.5 h (previous manual process) to 2.5 h. The number of enriched CD133$^+$ cells was $7.9x10^5$ (range: $3.7x10^5$ to $1.9x10^6$). The average yield was 47% and the average viability of the separated CD133$^+$ cells achieved 90% (range: 69.9% to 96.9%). The depletion of CD133 negative cells was >99.9%. CFU assays perfomed after the fully automated enrichment process showed that the CD133$^+$ cell fraction contained primitive and multipotent progenitor cells, such as CFU-GEMM and CFU-GM. The cell separation system described provides a safe and easy way to purify CD133$^+$ cells from bone marrow aspirates within 2.5 h without any intermediate manual steps. The cell preparation in a closed sterile system facilitates a fast and robust enrichment of CD133$^+$ cells. The cells are eluted in a small volume (6 mL) and can be used directly for further applications according to requirements e.g. for use in regenerative medicine.

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### Promotion of Wound Healing by Cord Blood Derived Unrestricted Somatic Stem Cells (USSCs) in a Murine Wound Healing Model and Analysis on Their Bio-Distribution by In Vivo Bioluminescent Imaging (BLI)

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**Background:** Delayed healing of skin wounds is a major morbidity. Repeated wounding is characteristic in patients with recessive dystrophic epidermolysis bullosa (RDEB), caused by mutations in *COL7A1* gene. Stem cell therapy offers an option in treating this disease (Kiru/Cairo et al. PNAS, 2011). Recently, cord blood (CB) derived pluripotent stem cells, USSCs, have been applied in several animal models of degenerative diseases with beneficial outcomes.

**Goal:** To determine the potential of USSCs in the treatment of RDEB and its associated wounding phenotype.

**Method:** CB-USSCs were characterized for genetic and functional properties. Their in vivo functions were evaluated in a murine full-thickness excisional wound healing model and by bioluminescent imaging (BLI), using USSCs modified with a luciferase reporter gene.

**Results:** CB-USSCs share several embryonic stem cell properties and could be induced to express hallmark genes of keratinocyte differentiation. USSCs constitutively express Col7A1, supporting their therapeutic potential in the treatment of patients with RDEB. In the wounding model, a single USSC intradermal injection promoted epithelialization and facilitated formation and remodeling of epidermis, accompanied by a significantly accelerated rate of wound healing on days 6-10 post wounding ($F_{(1,168)}=50.8$ $P<.01$). In vivo BLI revealed specific migration of USSCs from a distant intradermal injection site toward the wound, as well as following systemic injection. Temporal quantification on the total bioluminescence indicated an overall 59.9% signal loss over 3 days followed by a 95.06% loss at 1 week. The bioluminescence in the area of wound was then maintained at ~0.5-1% level till the end of the experiment (3 month). USSCs express several chemokine receptors that may mediate their migration to the wound, including CXCR4 (for SDF1), CCR7 (for CCL21) and PDGFRα (for HMGB1). In vitro chemotaxis assays indicated that SDF-1 significantly enhanced USSC migration at a concentration of 100ng/ml, while neither CCL21 nor HMGB1 showed significance even at a concentration of 10μg/ml. The effects of such chemokine/receptor interactions on USSC recruitment in vivo are now being investigated.

**Conclusion:** These results suggest significant beneficial effects of CB-USSCs on wound healing and raised the possibility of USSC's therapeutic beneifit in the treatment of patients with RDEB.

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### Another Method for Thawing Hematopoietic Stem Cells and its Impact in the Recovery of the Transplanted Hematological Patient

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**Introduction:** There are several hematopoietic-stem cells (HSCs) thawing methods for bone marrow reconstitution. They intend to avoid cell death and patient's side effects due to the dimethyl sulfoxide (DMSO). We propose another thawing method that diminishes cell death and therefore a more rapid hematological recovery.

**Material and Methods:** The standard thawing-removing DMSO method for cord blood units was described by